IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Mao *et al.*	EXAMINER: J.N. Fredman
SERIAL NO.: 09/867,201	ART UNIT: 1637
FILED: May 29, 2001	CONF. NO.: 5899
FOR: **SEQUENCING BY PROXY**	

Appeal Brief

Mail Stop Appeal Brief - Patents
Commissioner of Patents and Trademarks
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This brief is filed in support of an appeal to the Board of Appeals and Interferences from the decision of Examiner J.N. Fredman in the above-reference application, mailed September 27, 2006, in which claims 1-14 were finally rejected, and in view of the Advisory Action mailed February 27, 2007.

Appellants filed a Notice of Appeal on January 26, 2007. The present paper is Appellants' Appeal Brief submitted in compliance with 37 C.F.R. §1.192.

Also enclosed herewith are an Amendment under 37 CFR §41.33(b), a Petition for a two-month extension of time, and a check in the amount of $950, including the extension fee ($450) and the fee for filing this appeal brief ($500).

The Commissioner is hereby authorized and requested to charge any deficiency in fees herein to Deposit Account No. 50-2207.

REAL PARTY IN INTEREST

The real party in interest in this application is Solexa, Inc., the assignee of record, which was acquired by Illumina, Inc. on January 26, 2007. Under the merger agreement, Callisto Acquisition Corp., a wholly owned subsidiary of Illumina, merged with and into Solexa, with Solexa continuing as the surviving corporation. As a result of the merger, Solexa became a direct, wholly owned subsidiary of Illumina, Inc.

RELATED APPEALS AND INTERFERENCES

Appellants are not aware of other appeals or interferences which would directly affect, be directly affected by, or have a bearing on the Board's decision in the present appeal.

STATUS OF THE CLAIMS

The application was filed on May 29, 2001 with 16 claims. In a response to requirement for restriction and election of species, filed by the Appellants on February 27, 2006, claims 15-16 were withdrawn from consideration.

In the Amendment enclosed herewith, claims 1-2 and 4-8 are cancelled, claim 3 is redrafted in independent form, incorporating the subject matter of parent claims 1-2, and claim 9 is redrafted in independent form, incorporating the subject matter of parent claims 6-8.

In summary, claims 1-2 are cancelled, claim 3 is currently amended, claims 4-8 are cancelled, claim 9 is current amended, claims 10-14 are as originally filed, and claims 15-16 are withdrawn.

The claims involved in the appeal, assuming the entry of the current amendments (claims 3 and 9-14), are presented in Appendix I.

STATUS OF AMENDMENTS

All amendments submitted prior to the submission of this Appeal Brief have been considered and entered by the Examiner.

With respect to the enclosed amendment, 37 CFR §41.33(b) states:

Amendments filed on or after the date of filing a brief pursuant to §41.37 may be admitted:

(1) To cancel claims, where such cancellation does not affect the scope of any other pending claim in the proceeding, or

(2) To rewrite dependent claims into independent form.

Because the amendments currently submitted are limited to categories (1) and (2) above, Appellants have assumed that these amendments will be admitted and entered prior to consideration of this Appeal Brief.

SUMMARY OF THE CLAIMED SUBJECT MATTER

References to the Appellants' specification are based on the substitute specification filed on October 9, 2001.

Independent claim 3 provides a method of simultaneously determining a signature sequence of each polynucleotide in a sample of tag-polynucleotide conjugates. The method comprises the steps of:

(a) attaching an oligonucleotide tag from a repertoire of tags to each polynucleotide of the population to form tag-polynucleotide conjugates (*page 11, lines 5-33; page 14, line 4 to page 15, line 20*), such that substantially every different polynucleotide has a different oligonucleotide tag attached (*page 13, lines 1-6; page 16, lines 1-9*);

(b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated (*page 8, line 13 to page 9, line 25, especially page 9, lines 8-9; Fig 1a, ref. no. 120*);

(c) separating the polynucleotide fragments into size classes (*page 9, line 26 to page 10, line 2; page 10, lines 7-19; Fig 1b, ref. no. 154*);

wherein said steps of generating and separating include forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount, and wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease (*page 21, lines 11-13; page 21, line 30 to page 22, line 11; Fig. 2a, ref. no. 223, adaptors 226, restriction sites 228; Fig 2b*);

(d) labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of such polynucleotide fragment (*page 10, line 18 to page 11, line 2; page 22, lines 27-34; Fig 2d, ligation product 238, tag 206, labeled tags 246; page 24, lines 28-35; page 25, lines 9-15; Fig 3b, extended biotinylated strand 390, generation of labeled tags 395; Fig 4a, extension with biotinylated dNTP 420*);

(e) copying the labeled oligonucleotide tags of each polynucleotide fragment of each size class (*page 22, lines 27-34; Fig 2e, amplified tags 250; Fig. 3b, 395; Fig 4b, 426*); and

(f) separately hybridizing the labeled oligonucleotide tags of each size class with their

respective complements under stringent hybridization conditions, the respective complements being attached as populations of substantially identical oligonucleotides in spatially discrete and addressable regions on one or more solid phase supports, and the respective signature sequences being determined by the sequence of labels associated with each spatially discrete and addressable region of the one or more solid phase supports (*Fig 1b, 170, arrays 178-186, signals 190; page 19, line 18 to page 20, line 33; page 30, line 28 to page 31, line 26*).

Independent claim 9 provides a method of simultaneously determining a signature sequence of each polynucleotide in a sample of tag-polynucleotide conjugates, wherein substantially every different polynucleotide has a different tag attached. The method comprises the steps of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes (*page 8, line 13 to page 9, line 25, especially page 9, lines 8-9; Fig 1a, ref. no. 120*),

wherein said generating comprises: extending a first primer to copy said tag of each tag-polynucleotide conjugate, to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides (*page 25, line 22 to page 26, line 26; page 28, lines 7-11; Fig 4a-b*);

separating the size classes of polynucleotide fragments, wherein said separating includes forming substantially homogeneous populations of each of said size classes of said mixture by physical separation (*page 21, lines 8-10; page 28, lines 15-18*);

amplifying and labeling the tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of each such polynucleotide fragment (*page 10, line 18 to page 11, line 2; page 22, lines 27-34; Fig 2d, ligation product 238, tag 206, labeled tags 246; page 24, lines 28-35; page 25, lines 9-15; Fig 3b, extended biotinylated strand 390, generation of labeled tags 395; Fig 4a, extension with biotinylated dNTP 420; page 22, lines 27-34; Fig 2e, amplified tags 250; Fig. 3b, 395; Fig 4b, 426)*;

separately hybridizing the labeled tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached to each of a plurality of microarrays, each microarray of the plurality having the same spatially addressable hybridization sites; and determining each signature sequence in the

sample by a set of signals generated at hybridization sites having the same address on each of the plurality of microarrays (*Fig 1b, 170, arrays 178-186, signals 190; page 19, line 18 to page 20, line 33; page 30, line 28 to page 31, line 26.*

Dependent claims 10-14 recite specific embodiments of methods of carrying out physical separation of size classes in claim 9, and dependent claims 11-12 and 14 recite specific embodiments of the length and composition of the extension oligonucleotides of claim 9.

The role of "size ladders" and "size classes" in the claimed methods

In accordance with the claimed methods (see e.g. the embodiment of Fig. 1b), prior to sequencing the target polynucleotides, each is processed into a size ladder (see e.g. step b of claim 3). The size ladder for a given polynucleotide contains different-length portions of the sequence of that polynucleotide, each also containing the tag that corresponds to the original polynucleotide.

These size ladders for the population of polynucleotides are combined, and the resulting mixture of tagged fragments is separated into size classes (e.g. step c of claim 3). A size class will therefore contain differently tagged fragments from different polynucleotides, but the fragments within a class will be of a similar length, simplifying subsequent processing and sequencing of the fragments.

ISSUES (GROUNDS OF REJECTION TO BE REVIEWED)

The issues on appeal, in view of the rejections set forth in the final Office Action dated September 27, 2006 ("the Office Action"), are:

1. Whether claims 1, 4, 6-8 and 10 (or their pending counterparts) are unpatentable under 35 U.S.C. §102(b) as being anticipated by **Wong**, U.S. Patent No. 5,935,793 ("Wong").

2. Whether claims 1-12 and 14 (or their pending counterparts) are unpatentable under 35 U.S.C. §103(a) over **Brenner**, U.S. Patent No. 5,763,175 ("Brenner") in view of **Wong** (U.S. Patent No. 5,935,793).

3. Whether claim 13 is unpatentable under 35 U.S.C. §103(a) over **Brenner** (U.S. Patent No. 5,763,175) in view of **Wong** (U.S. Patent No. 5,935,793) and further in view of **Strathmann**, U.S. Patent No. 6,480,791 ("Strathmann").

SUMMARY OF APPELLANTS' ARGUMENTS

Regarding the rejections under 35 U.S.C. §**102(b)**, Appellants submit that the standard for anticipation is not met for the amended claims, because nowhere does Wong teach the claimed feature of:

forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount, wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease (independent claim 3);

nor does Wong teach the claimed feature of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes, wherein said generating comprises extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides (independent claim 9).

Regarding the rejections under 35 U.S.C. §**103(a)**, Appellants assert that the standard for obviousness has not been met, for the following reasons:

(1) Neither Wong nor Brenner teaches the claimed feature(s) of:

(b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated; and

(c) separating the polynucleotide fragments into size classes,

wherein said steps of generating and separating include forming a plurality of aliquots of tag-polynucleotide conjugates, and *shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot* such that said polynucleotides in different aliquots are shortened a different amount, and wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease (independent claim 3; emphasis added).

(2) Neither Wong nor Brenner teaches the claimed feature(s) of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes,

wherein said generating comprises: extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotide (independent claim 9, emphasis added).

(3) The Examiner's statement of motivation to combine these two references rests on interpretations of the terms "selection" and "sampling" which are inconsistent with their use in the cited references, as discussed further below.

APPELLANTS' ARGUMENTS

1. Rejection of Claims 1, 4, 6-8 and 10 under 35 U.S.C. §102(b) over U.S. Patent No. 5,935,793 (Wong)

Claim 1 and its dependent claims 4, 6-8 and 10 were rejected under 35 U.S.C. §**102(b)** as being anticipated by **Wong** (U.S. Patent No. 5,935,793).

A. The Cited Art

Wong describes a sequencing method in which a population of polynucleotides to be sequenced are provided with "identifier tags". The tagged polynucleotides are then processed according to conventional sequencing methods, typically by Sanger sequencing or Maxam-Gilbert sequencing (see column 17, lines 3-9 of the patent).

In the Maxam-Gilbert approach, as known in the art, the polynucleotide to be sequenced is cleaved specifically at a given nucleotide, for each of the four different nucleotides, generating a population of different-length fragments for each nucleotide. In the Sanger approach, as also known in the art, a polynucleotide to be sequenced is used as a template for replication, and a given terminating nucleotide, such as a dideoxynucleotide, is used to generate a series of different-length fragments terminating in that nucleotide.

The different-length fragments so produced are separated, typically by electrophoresis. In

the method of Wong, each fragment can then be correlated with the polynucleotide from which it is derived, by way of the above-noted "identifier tags". Thus, a population of polynucleotides can be sequenced simultaneously on the same electrophoresis medium.

B. The Rejections

Of the rejected claims, claims 1, 4, and 6-8 are cancelled by the enclosed amendment. Therefore, the rejection of these claims is moot.

Claim 3

Independent claim 3 incorporates the limitations of previous claims 1, 2, and 3. Because previous claims 2 and 3 were not rejected under this section, it is assumed that the Examiner did not find that Wong teaches the limitations of claims 2 and 3. Specifically, Wong does not teach the claimed feature(s) of:

forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount, wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease (independent claim 3).

Claims 9 and 10

Independent claim 9 and dependent claim 10 incorporate the limitations of previous claims 6, 7, 8, and 9. Because previous claim 9 was not rejected under this section, it is assumed that the Examiner did not find that Wong teaches the limitations of claim 9. Specifically, Wong does not teach the claimed feature(s) of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes, wherein said generating comprises extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides (independent claim 9).

Thus, Appellants urge the Board to reverse the rejections under 35 U.S.C. §102(b).

2. Rejection of Claims 1-12 and 14 under 35 U.S.C. §103(a) over U.S. Patent No. 5,763,175 (Brenner) and U.S. Patent No. 5,935,793 (Wong)

Claims 1-12 and 14 were rejected under 35 U.S.C. §**103(a)** over **Brenner** (U.S. Patent No. 5,763,175) in view of **Wong** (U.S. Patent No. 5,935,793).

Of these claims, claims 1-2 and 4-8 are cancelled by the enclosed amendment. Therefore, the rejection of these claims is moot.

As stated above, independent claim 3 incorporates the limitations of previous claims 1-3, and independent claim 9 incorporates the limitations of previous claims 6-9. Dependent claims 10-12 and 14 also incorporate the limitations of previous claims 6-9.

A. Rejection of Claim 3

With regard to the subject matter of current claim 3 (which combines the subject matter of previous claims 1-3), the Examiner asserted (page 6 of Office Action) that **Brenner** teaches the subject matter of previous claim 1, with the exception of the step(s) of "generating a size ladder and separating the polynucleotides into size classes" (steps b-c of previous claim 1) (Office Action, top of page 7). The Examiner asserted that **Wong** teaches these steps of "generating a size ladder" and "separating the polynucleotides fragments into size classes" (Office Action, page 7).

The Examiner also asserted (Office Action, page 6) that, with regard to "claims 2-3, 9, **Brenner** teaches where the type IIS enzyme Bbv I is used to shorten (column 21, lines 29-45)".

With respect to this last assertion by the Examiner, the Appellants submit that Brenner does not teach the claimed steps of:

(b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated;

(c) separating the polynucleotide fragments into size classes,

wherein said steps of generating and separating include forming a plurality of aliquots of tag-polynucleotide conjugates, and *shortening by a different amount said polynucleotides of*

said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount, and wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease (independent claim 3; emphasis added).

As discussed further below in Section B, cleavage of a target polynucleotide with the type IIS enzyme (Bbv I) in Brenner, mediated by an attached adaptor, cleaves at the same position in the attached polynucleotide, relative to the attached adaptor, for each polynucleotide. Therefore, this process does not constitute "*shortening by a different amount* said polynucleotides of said tag-polynucleotide conjugates".

B. The Cited Art

Brenner (U.S. Patent No. 5,763,175) is directed to a method of simultaneous sequencing of polynucleotides. As shown in the embodiment of Fig. 2 of Brenner, multiple sets of "S primers" are applied to a target construct which includes, at one terminus, an S primer binding site (22), which is adjacent to the target polynucleotide (20). The S primers are provided in sets of four, as shown in the Figure, where within each set one nucleotide is either A, G, C, or T. The different sets of S primers (shown as Sets 1, 2, 3, ...k) differ in how far this varying nucleotide, which is the site of sequence interrogation, extends into the target polynucleotide.

The S primers in Brenner also contain a IIS restriction enzyme recognition site, so that, after a round of sequencing, the end of the polynucleotide which has been sequenced can be removed by enzymatic cleavage.

Exemplary sets of S primers are shown at column 20, lines 5-15 of the patent, which refers to the "following 32 S primers". Each of the eight structures shown therefore represents a set of four S primers (for a total of 32), in accordance with Figure 2. Each structure includes: a 3' interrogating region containing the variable nucleotide X, the IIS recognition site (Bbv I site), and an adjacent region which is identical for each primer (AAAAAGGAGGAGG in this set of primers). This identical region must contain the primer binding region; therefore, the position of the BBv I site is constant with respect to the primer binding region. Consequently, the enzyme will cleave at the same position in the target polynucleotide for each primer, thus shortening each target polynucleotide by the same amount. This is also reflected at column 14, lines 41-44 of the patent: "...the nuclease will

be positioned by the recognition site to cleave the target polynucleotide a predetermined number of nucleotides from the border of the S primer binding site".

Therefore, as stated above, this process does not constitute "*shortening by a different amount* said polynucleotides of said tag-polynucleotide conjugates", as recited in Appellants' claim 3.

(With further reference to Figure 2 in Brenner, Appellants note that the Figure shows amplicons of different apparent lengths, which could at first glance suggest a "size ladder". However, these length differences apparently result from the different lengths of the 5' extensions on the primers used for amplification, as shown in the poly-I regions of the primer sets at column 20, lines 5-15. They do not result from enzymatic cleavage.)

Because previous claims 2 and 3 were not rejected under 35 U.S.C. §102(b), it is assumed that the Examiner did not find that **Wong** taught the limitations of claims 2 and 3. More specifically, Wong does not teach the claimed feature(s) of:

forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount, wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease.

Because **neither Wong nor Brenner** teaches or suggests this feature of independent claim 3, the claim cannot be found unpatentable over these references. (To establish a *prima facie* case of obviousness, "...the prior art reference, or references when combined, must teach or suggest all the claim limitations." See MPEP §2143.)

C. Rejection of Claim 9 and Dependent Claims 10-12 and 14

With regard to the subject matter of current claim 9 (which combines the subject matter of previous claims 6-9), the Examiner asserted that: **Wong** teaches the subject matter of previous claim 6; "with regard to claim 7, **Wong** teaches formation of a size ladder for each tag-polynucleotide (see column 17, line 20 to column 18, line 59 and see column 26, claim 1, step (c))"; "with regard to claims 8, 10, **Wong** teaches physical separation using

electrophoretic methods..."; and "with regard to claims 2-3, 9 [sic], **Brenner** teaches where the type IIS enzyme Bbv I is used to shorten (column 21, lines 29-45)".

Appellants note that the subject matter of previous claim 9 does not pertain to the use of type IIS enzymes for shortening; therefore, the Examiner's characterization of previous claim 9 appears to be misplaced in this instance.

Claim 9 in fact includes the limitation of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes,

wherein said generating comprises: extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides.

The Examiner did refer to the similar subject matter of claims 4-5 (now cancelled) as also being taught by **Brenner**: "With regard to claims 4-5, Brenner teaches wherein extension products of known length are ligated onto each tag-polynucleotide (see column 21, lines 38-45, where S primers are ligated to the cleaved tag-conjugates)" (page 6 of Office Action). However, for the reasons described below in Section D, this passage does not teach or suggest the subject matter of claim 9.

D. The Cited Art

Upon review of the passage in **Brenner** cited by the Examiner (column 21, lines 38-45), it is seen that what the Examiner refers to as "extension products of known length" (extension oligonucleotides) are not "S primers" but rather an "adaptor mixture containing a new S primer binding site" (column 21, lines 46-47).

Appellants note that pending claim 9 recites "ligating extension oligonucleotides", in the plural. As described at page 26, lines 14-16 of Appellants' specification, such ligation of "oligonucleotides", in the plural, creates "a set of polynucleotide fragments...each differing in length from one another by integral multiples of the length of the extension oligonucleotide" (i.e. a "size ladder").

Brenner, on the other hand, teaches the ligation of a single, same-length adaptor onto each cleaved polynucleotide target (column 21, lines 38-45, as cited by the Examiner), which would not generate a "size ladder" of polynucleotide fragments as recited in the claim.

Because previous claim 9 was not rejected under 35 U.S.C. §102(b), it is assumed that the Examiner did not find that **Wong** taught the limitations of claim 9. Specifically, Wong does not teach the claimed feature(s) of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes, wherein said generating comprises extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides (independent claim 9).

Because **neither Wong nor Brenner** teaches or suggests this feature of independent claim 9, this claim and its dependent claims 10-14 cannot be found unpatentable over these references. (To establish a *prima facie* case of obviousness, "...the prior art reference, or references when combined, must teach or suggest all the claim limitations." See MPEP §2143.)

Accordingly, Appellants submit that the appealed claims 3 and 9-14, as amended, patentably define over the teachings of Wong and Brenner, and ask that the Examiner's rejections under 35 U.S.C. §103(a) be reversed.

E. Combination of References

Appellants note that, as demonstrated above, the cited references, even when combined, do not teach or suggest all of the limitations of the independent claims. Therefore, a showing of motivation to combine would not defeat the patentability of the claims over these references. In any event, the Appellants find the motivation provided by the Examiner to be misdirected.

The Examiner suggests that one would be motivated to modify Brenner by "using the size

selection of Wong", to "achieve a sampling frequency...useful for shotgun sequencing" (pages 11-12 of Office Action).

The passage pointed to in Wong by the Examiner (column 12, lines 6-16) stipulates the length of polynucleotide fragments (i.e., 400-2000 nucleotides) that should be used in a sequencing operation "to achieve a desired sampling frequency for effective shotgun sequencing". One skilled in the art would understand this passage to mean that sequencing the end regions of fragments of this length should provide a satisfactory representation of the original polynucleotide in "shotgun sequencing".

The passage pointed to in Brenner by the Examiner (column 11, lines 50-55) concerns the number of tag-polynucleotide conjugates that should be selected ("sampled") from a large population of tag-polynucleotide conjugates, in order to reduce the probability of different polynucleotides having the same tag ("doubles"), while still using a large enough sample of the original population of tag-polynucleotide conjugates for "adequate coverage of a target polynucleotide in a shotgun sequencing operation."

The "sampling frequencies" referred to in these two passages, then, refer to entirely different concepts. Thus, the "size selection of Wong" (i.e. polynucleotide fragments 400-2000 nucleotides in length) has no relevance to "achieving a sampling frequency" as taught in Brenner. The process of "sampling" in Brenner refers to selecting a sample of tag-polynucleotide conjugates from a larger population of tag-polynucleotide conjugates, and is not concerned with the length of the polynucleotides.

3. Rejection of Claim 13 under 35 U.S.C. §103(a) over U.S. Patent No. 5,763,175 (Brenner) and U.S. Patent No. 5,935,793 (Wong) and U.S. Patent No. 5,935,793 (Strathmann)

Claim 13 was rejected under 35 U.S.C. §**103(a)** over **Brenner** (U.S. Patent No. 5,763,175) in view of **Wong** (U.S. Patent No. 5,935,793) and further in view of **Strathmann**, U.S. Patent No. 6,480,791 ("Strathmann").

Dependent claim 13 further limits dependent claim 12 by reciting that size classes of polynucleotides are separated by denaturing HPLC.

The Examiner notes that Strathmann teaches that "tagged polynucleotides can be separated

by HPLC" (page 10 of Office Action).

Claim 13 is ultimately dependent on independent claim 9 and contains all of its limitations. As discussed above, Wong and Brenner, taken alone or in combination, do not teach all of the features of independent claim 9. The Strathmann patent does not make up for the deficiencies of these references.

Accordingly, Appellants submit that the appealed claims 3 and 9-14, as amended, patentably define over the teachings of Wong, Brenner, and Strathmann and ask that the Examiner's rejections under 35 U.S.C. §103(a) be reversed.

CONCLUSIONS

In view of the foregoing discussion, Appellants submit that the pending claims are in condition for allowance and patentably define over the prior art, and urge the Board to overturn the Examiner's rejections.

Respectfully submitted,

Date: May 24, 2007

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APPENDIX I: CLAIMS ON APPEAL

3. A method of simultaneously determining a signature sequence for each polynucleotide in a population of polynucleotides, the method comprising the steps of:

(a) attaching an oligonucleotide tag from a repertoire of tags to each polynucleotide of the population to form tag-polynucleotide conjugates, such that substantially every different polynucleotide has a different oligonucleotide tag attached;

(b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated;

(c) separating the polynucleotide fragments into size classes,

wherein said steps of generating and separating include forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount, and wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease;

(d) labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of such polynucleotide fragment;

(e) copying the labeled oligonucleotide tags of each polynucleotide fragment of each size class; and

(f) separately hybridizing the labeled oligonucleotide tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached as populations of substantially identical oligonucleotides in spatially discrete and addressable regions on one or more solid phase supports, and the respective signature

sequences being determined by the sequence of labels associated with each spatially discrete and addressable region of the one or more solid phase supports.

9. A method of simultaneously determining a signature sequence of each polynucleotide in a sample of tag-polynucleotide conjugates, wherein substantially every different polynucleotide has a different tag, the method comprising the steps of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes,

wherein said step of generating includes: extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides;

separating the size classes of polynucleotide fragments, wherein said separating includes forming substantially homogeneous populations of each of said size classes of said mixture by physical separation;

amplifying and labeling the tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of each such polynucleotide fragment;

separately hybridizing the labeled tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached to each of a plurality of microarrays, each microarray of the plurality having the same spatially addressable hybridization sites; and

determining each signature sequence in the sample by a set of signals generated at hybridization sites having the same address on each of the plurality of microarrays.

10. The method of claim 9, wherein said step of forming by said physical separation is carried out by preparative gel electrophoresis or HPLC.

11. The method of claim 10, wherein said extension oligonucleotides have a length of from 2 to 10 nucleotides.

12. The method of claim 11, wherein said extension oligonucleotides have a length of from 4 to 6 nucleotides.

13. The method of claim 12, wherein said step of forming by said physical separation is carried out by denaturing HPLC.

14. The method of claim 13, wherein said extension oligonucleotides comprising one or more degeneracy-reducing nucleotide analogs.

APPENDIX II: EVIDENCE

None

APPENDIX III: RELATED PROCEEDINGS

None